REMARKS

Entry of the above amendments and consideration of the following remarks are respectfully requested. Upon entry of the above amendments, this application will contain claims 1-20 pending and under consideration. As more fully discussed below, Applicants believe that the claimed invention is patentably distinct over the cited references and respectfully request reconsideration leading to withdraw of all outstanding rejections and allowance of this application in a timely fashion.

The specification was objected to because of informalities. In response, Applicants have inserted section headings in the appropriate locations throughout the written specification. Applicants believe that this objection is overcome. Additionally on page 19 immediately after the word "line" the number --1-- was added.

Claim 20 was amended to correct minor typographical errors, specifically on line 7 of the claim after the word "complementary", the phrase --3' binding region-- was added. It is believed that no new matter is entered by these amendments.

Claims 1-20 stand rejected under 35 U.S.C. § 103(a) over Fraiser et al. (U.S. 5,648,211) in view of Cleuziat et al. (U.S. 5,824,517).

Applicants urge that the combination of Fraiser and Cleuziat do not make the claimed invention obvious. These references cannot be combined to make the claimed invention obvious because modification of the method described in Fraiser et al. according to Cleuziat et al. would render Fraiser's Strand Displacement Amplification (SDA) inoperable for its intended purpose. This obviously negates any motivation for one skilled in the art to combine teachings found in these two references to arrive at the claimed invention.

Applicants' claimed invention requires the use of an enzyme having a 5'-3' double strand specific exonuclease activity. The exonuclease polymerase digests a portion of the hybridized, long, first primer, either AB primer or CD primer, and exposes a binding site on a double stranded DNA. A short, second primer, either an A primer or a C primer, can bind at the exposed site. Subsequently, the enzyme with strand displacing polymerase activity can initiate extension and strand displacement polymerization starting with the short hybridized primers A or C. The displaced strand, with the "partially digested" long primer can then recycle back through the process to produce two more double stranded molecules. (application page 10, line 7 through

page 12, line 3, and FIGs. 3d-3m.) Consequently, without the exonuclease polymerase amplification of the targeted gene sequence could not be accomplished.

The Examiner noted that Fraiser et al does not disclose a T7 Gene 6 exonuclease, which is correct as far as the statement goes. Fraiser et al. does not disclose this enzyme because Frasier et al. actually describes a strand displacement amplification technique, which requires a polymerase that specifically lacks 5'-3' exonuclease activity. (Fraiser et al., col. 6, lines 18-22.) Fraiser et al. further adds that if a polymerase that has 5'-3' exonuclease activity is present, this exonuclease activity must be inactivated before use in the SDA method. (Fraiser et al., col. 6, lines 45-61.) Considering Fraiser's SDA process, an enzyme that has 5'-3' exonuclease activity would inhibit the amplification. If present, the 5'-3' exonuclease active enzyme would digest the polymerized double stranded section including the sequence targeted for amplification. Exonuclease polymerase digestion would compete with primer extension and displacement by the exonuclease inactive polymerase on the doubled stranded molecules. (See Fraiser, et al., col. 4, line 45 -col. 5, line 64 and Fig. 2.)

At best Cleuziat et al. merely mentions that a DNA polymerase having an exonuclease 5'-3' activity can be used when it does not prevent the amplification process from being carried out. (Cleuziat et al., col. 9, lines 56-58.) Clearly as we noted above, addition of an enzyme with exonuclease activity would prevent the Fraiser's SDA amplification process, and Fraiser et al. specifically requires that this enzyme either not be used or that its 5'-3' exonuclease activity be inactivated. Therefore to modify the method described in Fraiser et al. using the Cleuziat et al.'s process with a polymerase having 5'-3' exonuclease activity would render Fraiser's SDA process inoperable. One skilled in the art would not be motivated to combine the teachings of Fraiser et al. with those of Cleuziat et al. because to do so would render Fraiser's SDA process inoperable for its intended purpose.

Additionally Cleuziat's method utilizes chimeric DNA/RNA primers in which the RNA portion is represented in the Figures as a wavy line. The reaction conditions include an enzyme system with polymerase activity, strand displacement activity and RNAse H activity. The polymerase preferable does not have the exonuclease activity. (Cleuziat et al., col. 9, lines 46-62.) The manner in which the amplification is effected using the DNA/RNA primers is illustrated in FIG. 5. It will be seen that the chimeric DNA/RNA primer A1 hybridises to a strand 12 that was generated earlier in the reaction, for example, as disclosed in FIG. 1. The

primer A1 is extended to produce strand 18 as illustrated for the second step of FIG. 5. The RNA portion of the extended or even extending strand is digested by the RNAse enzyme to "expose" a site in which the primer B1 may find. This primer, B1, is self-extending and causes displacement of the strand 19. As depicted in FIG. 5, the reaction continues with generation of sites at which RNA provided by a primer A1 or A2 is hybridised to DNA, then allowing the RNA to be digested to expose a primer binding site.

In contrast the claimed invention does not require the use of an RNA portion or RNAse H enzyme to digest a portion of the RNA/DNA molecule to expose a binding site for a primer, The present invention uses an enzyme having 5'-3' exonuclease activity. The present invention provides significant advantages over Cleuziat's method. Cleuziat's method requires RNA which are extremely susceptible to degradation by ribonucleases that are common in DNA preparations. (Application, page 3, lines 4-18.) These degradation reactions obviously siphon off otherwise usable molecules into unproductive side products and can significantly reduce amplification yield and efficiency. Since the claimed invention does not require any RNAe or RNAse H, these degradation problems are circumvented.

Applicants respectfully suggest that Fraiser et al. and Cleuziat et al. either singly, or in combination, do not disclose or make obvious the claimed invention. Accordingly, reconsideration leading to withdraw of all the rejections under 35 U.S.C. § 103(a) and allowance of this application containing claims 1-20 are respectfully requested. Additionally, the Examiner is invited to telephone the undersigned attorney if there are any questions about this submission or other formal matters, which may be addressed in that fashion.

Respectfully submitted,

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ADDENDUM

In the Specification

--The results are shown in Fig. 5 in which lane 1 is a 100bp marker and lanes 2-7 respectively illustrate the results obtained at "0 time" 60, 90, 120, 150 and 180 minutes. The production of amplified product is clearly shown in lanes 5-7.--

In the Claims

20. A method of amplifying complementary first and second nucleic acid sequences each of which has a binding region at its 3' end, the method comprising treating the separated single stranded sequences with

(a) first and second primers each capable of hybridising to the 3'-binding regions of the first and second strands respectively and each including remote from its 5'-end a digestion resistant region which, with the primer hybridised to a complementary 3'-binding region, allows only partial digestion of the primer by the enzyme (d) having 5'-double strand specific exonuclease activity,

(b) third and fourth primers each having a degree of sequence homology with the particularly digestible regions of the first and second primers respectively whereby the third and fourth primers are capable of hybridising to the 3'-binding regions of the first and second strands respectively,

(c) an enzyme having strand displacing polymerase activity,

(d) an enzyme having 5' double stranded specific exonuclease activity, said enzyme (d) possibly being provided by enzyme (c) in the case where the latter also has the required exonuclease activity, and

(e) nucleoside triphosphates, at least a portion of at least one of which is modified such that when it is incorporated into a growing nucleic it is resistant to digestion by the exonuclease.

under conditions permitting hybridisation, exonuclease digestion and strand displacement polymerisation thereby producing an amplified amount of the first and second strands.